

CLINICAL IMPLICATIONS OF BASIC RESEARCH

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Asthma is characterized by chronic inflammation of the airways, contraction of smooth muscle, overproduction of mucus, and remodeling of the airway wall. In many forms of asthma, there is an accumulation of eosinophils, which predicts a good response to inhaled glucocorticoids. Eosinophilic inflammation is controlled by type 2 helper T (Th2) lymphocytes of the adaptive immune system, which make interleukin-4, interleukin-5, and interleukin-13 in response to the presentation of an allergen by dendritic cells in the airway. However, many features of asthma can also be controlled by type 2 innate lymphoid cells, which cannot specifically recognize allergens but do produce interleukin-5 and interleukin-13 when stimulated by cytokines made by lung epithelial cells and macrophages.

Although immune cells control all the features of asthma, it is unclear why and how they react to allergens. Most organisms that express allergens, such as pollinating plants, house-dust mites, cockroaches, and environmental fungi, are not intrinsically pathogenic to the host. However, some proteins or lipids of these organisms trigger pattern-recognition receptors of the mammalian immune system. For example, the house-dust mite is known to carry at least 20 allergens that can induce IgE responses in humans. In mice, the development of an asthmalike condition triggered by house-dust mites depends on the expression of toll-like receptor 4 (TLR4) on airway epithelial cells.¹ Although many allergens contain lipopolysaccharide, which can potentially bind and activate TLR4, it is also possible that TLR4 is triggered by endogenous danger signals that are released on exposure to allergens.

Some allergens have proteolytic activity and trigger protease-activated receptors on barrier epithelial cells and dendritic cells, but until the recent publication of an article by Millien et al.,² there was no known link between protease allergens and TLR4 signaling. Millien et al. found

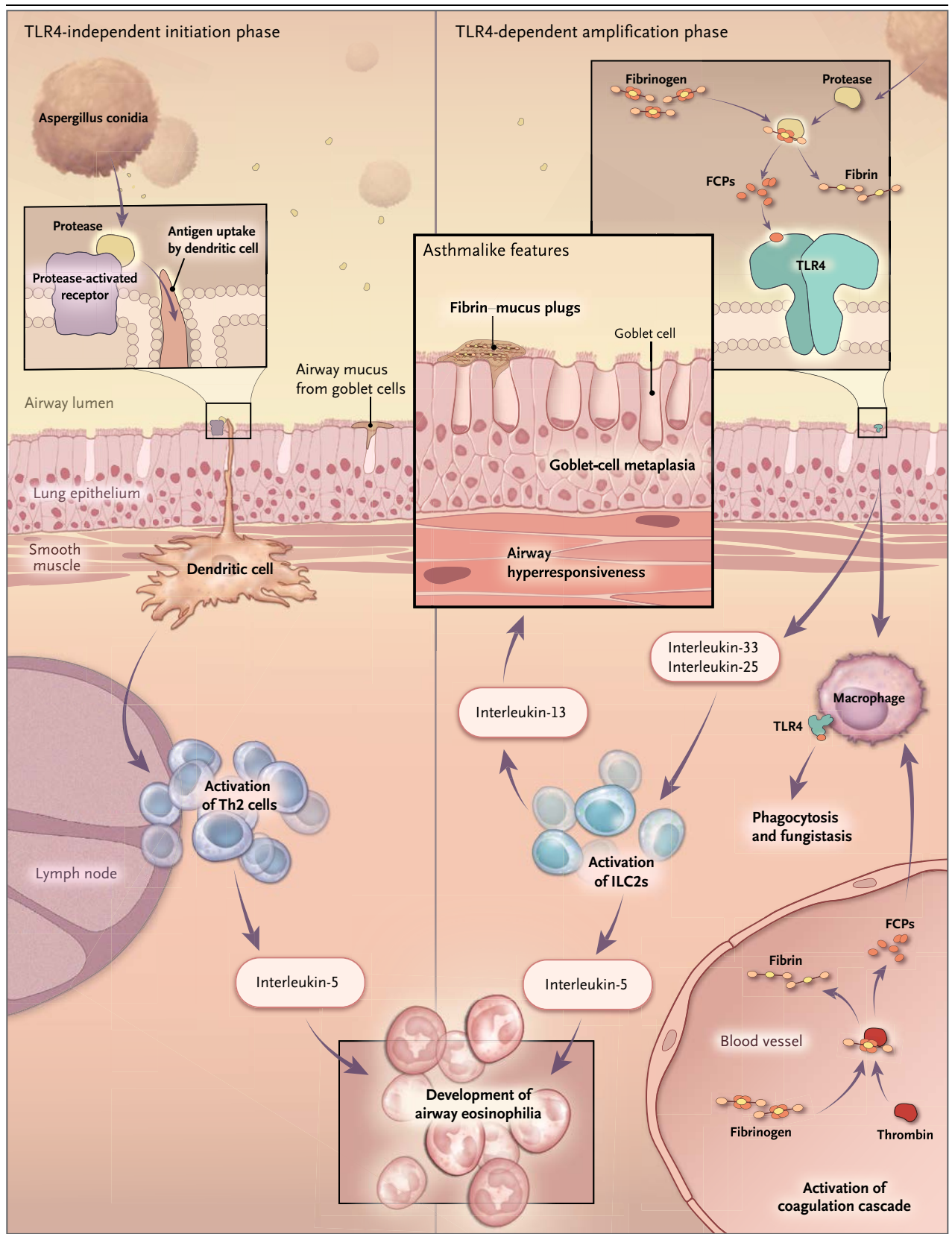
that TLR4 has a critical role in the allergic response to fungal proteases, such as those derived from environmental aspergillus species.

Colonization by aspergillus is frequently found in patients with severe asthma, and it causes allergic bronchopulmonary aspergillosis, a syndrome characterized by eosinophilic pulmonary infiltrates, high production of IgE, and central bronchiectasis. Millien and colleagues found that the development of airway inflammation, mucus hypersecretion, and bronchial hyperreactivity in response to the inhalation of an aspergillus protease depends on TLR4 and its downstream adaptor molecules (Fig. 1). As compared with wild-type control mice, TLR4-deficient mice, on exposure to aspergillus protease, showed a paltry recruitment of type 2 innate lymphoid cells (despite normal numbers of Th2 cells) and a reduction in asthmalike features.

Millien et al. also found that the activation of TLR4 in wild-type mice by a fungal protease was indirect and required the presence of a serum factor. While trying to identify the responsible

Figure 1 (facing page). Fibrinogen Cleavage Products in Asthma.

When fungal spores or proteases from aspergillus are inhaled, they can trigger the activation of dendritic cells that cause the development of type 2 helper T (Th2) immunity in the draining lymph nodes. Th2 lymphocytes then return to the lungs to cause mild eosinophilia and mucus production by goblet cells. Millien and colleagues² found that fungal proteases subsequently cleave fibrinogen into fibrin and fibrinogen cleavage products (FCPs). The FCPs trigger the toll-like receptor 4 (TLR4) on epithelial cells to cause the recruitment of type 2 innate lymphoid cells (ILC2s), which further boost airway eosinophilia and mucus production. The fibrin assembles with mucus to form plugs that can obstruct the airways. At the same time, TLR4 triggering by FCPs causes macrophages to inhibit fungal growth. This system in which a molecule involved in coagulation activates TLR4 is reminiscent of the way Toll is activated by Spätzle in fruit flies.



serum component, the authors considered how toll-like receptors were discovered in the model organism *Drosophila*. Mutations in the genes *Toll* and *Spätzle* caused fruit flies to be colonized by *Aspergillus fumigatus*.³ The Toll ligand pro-Spätzle is activated by a serine protease — a mechanism that also underlies the activation of coagulogen, a clotting factor of the horseshoe crab, an invertebrate that has changed little over the past 400 million years.⁴ Millien et al. found that, similarly, fungal proteases cleave the serum factor fibrinogen, thus causing clot formation and releasing fibrinogen cleavage products that can activate TLR4. The activation of TLR4 by fibrinogen cleavage products subsequently triggers antifungal immunity and fungistasis of *Aspergillus* conidia grown on alveolar macrophages, just as it does in *Drosophila*.

Thrombin, the classic activator of coagulation, also generates fibrinogen cleavage products from fibrinogen, thus triggering TLR4. The thrombin inhibitor hirudin was able to suppress aspergillus-driven asthmalike changes in mice, but it also suppressed asthmalike changes driven by the model antigen ovalbumin, which has no protease activity.² Allergen challenge in humans and mice is generally accompanied by the extravasation of plasma, platelet aggregation, and activation of the coagulation cascade in the lung interstitium and bronchoalveolar compartment, with elevations of tissue factor, thrombin, and fibrinogen, which explains how fibrinogen cleavage products can be generated with most allergens.⁵

Millien et al. found that activation of the coagulation cascade by endogenous or allergen-derived proteases is an important factor in driving asthmalike changes in mice by means of

TLR4 signaling. Additional research is required to evaluate whether this finding can be translated into clinical applications. The fibrinogen cleavage products need to be defined more precisely before drug candidates can be screened. It remains to be determined whether fibrinogen cleavage products are present in the airways of persons with asthma. Although Millien et al. were able to suppress aspergillus-induced asthmalike changes by blocking the generation of fibrinogen cleavage products, it is notable that trials of inhaled (low-molecular-weight) heparin in the context of allergen-challenge studies involving humans have yielded various effects. Given the heterogeneous nature of the disease, it is conceivable that a subset of patients with asthma may one day benefit from antithrombotic medications.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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1. Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009;15:410-6.
2. Millien VO, Lu W, Shaw J, et al. Cleavage of fibrinogen by proteinases elicits allergic responses through Toll-like receptor 4. *Science* 2013;341:792-6.
3. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette *spätzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell* 1996;86:973-83.
4. Arnot CJ, Gay NJ, Gangloff M. Molecular mechanism that induces activation of Spätzle, the ligand for the *Drosophila* Toll receptor. *J Biol Chem* 2010;285:19502-9.
5. de Boer JD, Majoor CJ, van 't Veer C, Bel EH, van der Poll T. Asthma and coagulation. *Blood* 2012;119:3236-44.

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